SOUTHERN CALIFORNIA PARTICLE CENTER AND SUPERSITE (SCPCS)
Progress report

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Overview of the Southern California PM Center:
The overall objective of the Southern California Particle Center and Supersite (SCPCS) is to bring together outstanding scientists from the leading universities in Southern California to identify and conduct high priority research to better understand the effects of particulate matter (PM) and ensure protection of public health. The SCPCS makes use of an integrated approach to address each of the areas of Exposure, Dosimetry, Toxicology, and Epidemiology that are identified in EPA's RFA. This integration is accomplished by seeking out and involving in the Center some of the major figures in air quality and environmental health. A team has been assembled for SCPCS that is committed to the need for strong cross- and interdisciplinary programs of research in order to address the challenging issues posed by PM.

The Los Angeles Basin is home to more than 14 million individuals who breathe the most polluted air in the nation. The research projects in this Center will provide the airshed with a detailed characterization by exposure studies to assess PM size, composition, and spatial variability. Toxicology investigations are directed toward a fundamental understanding of the mechanisms and the chemical and physical components of PM. Epidemiologic research is likely to provide new information to enhance our ability to detect human health effects from ambient air pollution.

Supplemental Keywords: Airborne particulate matter, aerosol, size distribution, particle concentrator, NRC priorities, mechanism, quinones, allergens, bioaerosols, dosimetry, children's study, indoor exposure, exposure assessment, ultrafine, fine and coarse particles, REHEX, asthma, PAHs, clinical human exposures, source-receptor, measurement error, study design, susceptible populations, geo-code, toxicology, epidemiology, regional modeling, source/receptor analysis, Southern California, Los Angeles basin, photochemistry, meteorology, trajectory modeling, peroxides.

Relevant Web Sites: www.scpcs.ucla.edu

I. Principal Theme of the SCPCS

The principal theme of the Southern California Particle Center and Supersite (SCPCS) is **"Mobile Source Pollution and Health Effects"** (Figures 1 and 2). All of the research within the SCPCS is intended to address specific priorities of the National Research Council (NRC).

The funding from EPA has brought together faculty from UCLA, USC, UC Riverside, UC Davis, UC Irvine, Rancho Los Amigos, and Michigan State University. Participating faculty within the SCPCS comprise a wide range of disciplines including toxicology, epidemiology, biostatistics, immunology, pharmacology, medicine, atmospheric sciences, atmospheric and environmental chemistry, exposure assessment, and aerosol science. The challenge to the SCPCS has been to bring together a wide range of expertise and the success of the Center has been our ability to link disciplines and researchers to a well defined set of research activities. That linkage also includes interaction and funding from the California Air Resources Board (ARB) and the South Coast Air Quality Management District (SCAQMD). Given the extremely poor air quality in Southern California, it is unfortunate that a Center similar to SCPCS had not been established many years ago. There is a continuing need for this Center in the future. To date, the SCPCS has generated a wide range of projects and has produced important findings and data within the overall theme and the hypotheses described below.

II. SCPCS research activities

A second key theme also guides the research of the Center: "Identification of the important physical/chemical characteristics of airborne PM responsible for the adverse health effects associated with PM and co-pollutant exposures."

A key feature of the research in the SCPCS is the linkage between exposure assessment/PM characterization and toxicological and human health outcomes. All health studies involve simultaneous exposure assessment/PM characterization.

The SCPCS research programs may be subdivided into three general areas:

A. Studies emphasizing investigation of the biological mechanisms of PM effects in relation to PM physical and chemical characteristics;

B. Studies of emission sources and related adverse health effects;

C. Varying spatial and temporal patterns of ambient PM and co-pollutants and resulting health effects; particular emphasis on the role of atmospheric chemistry.

All studies in the SCPCS are being conducted within the context of the secondary theme and "the investigation of the biological mechanisms of PM effects". Research areas B and C are complementary and fundamentally linked with A. All three focus on the role of organic and metal constituents of PM (see first hypothesis below). The research progress will be summarized in the sections below, but additional research findings are also described in more detail in the SCPCS report to the External Science Advisory Committee and the most recent report on our

Supersite activities. Research activities underway in the Supersite are an important complement to the overall SCPCS activities. This report is included under separate cover. Publications derived from SCPCS research are listed at the end of the text of this report (pp. 22-25). All studies in the SCPCS are enhanced by the availability of ultrafine, fine and coarse mobile concentrators which are linked to in vitro sample collection, in vivo studies, and human clinical studies described in this report. Figure 3 illustrates the sites for field studies being conducted in the LAB.

A. Studies emphasizing investigation of the biological mechanisms of PM effects in relation to PM physical and chemical characteristics

Hypothesis – A central hypothesis of the SCPCS

?? **Organic constituents associated with PM, including quinones, other organic compounds (PAHs, nitro-PAHs, and aldehydes/ketones) and metals, are capable of generating reactive oxygen species (ROS) and acting as electrophilic agents. They have a central role in allergic airway disease including asthma and cardiovascular effects through their ability to generate oxidative stress, inflammation and immunomodulating effects in the lungs and airways.**

The two mechanisms of oxidative stress and electrophilic addition result in impairment and damage to respiratory and cardiac functions. (See Appendix I for further discussion.) Catalytic action by quinones and related organic compounds and metals represents a key pathway in the toxicity of PM. Quinones produce futile redox cycles as long as reducing equivalents are available thus enabling continual production of O_2^-, H_2O_2 and OH radical. Macrophages and epithelial cells are the principal targets of ROS and the electrophilic agents. We propose the mechanistic features of the toxicity include activation of NF-kB and AP-1 pathways leading to cytokine and chemokine production and subsequent inflammatory responses, effects on mitochondrial function and apoptosis (Figure 4). In addition, epidemiologic studies have suggested an association of ambient particulate matter such as PM2.5 with cardiopulmonary diseases and mortality. 9,10-Phenanthroquinone (9,10-PQ) is a potent inhibitor of neuronal form of nitric oxide synthase (NOS). 9,10-PQ also inhibits the endothelial form of NOS which plays a critical role in vascular tone, thereby causing the suppression of NO-dependent vasorelaxation of aorta and significant increase in blood pressure in rats. Therefore, quinones and other compounds producing ROS, e.g., nitro-PAHs may contribute to diseases related to vascular dysfunction caused by exposure to urban air particles. In addition to the production of ROS, quinones, PAHs, nitro-PAHs, and related compounds may also undergo electrophilic addition to macromolecules producing complementary toxicity.

Within this mechanistic context we have defined four dosimetric models that represent hypothetical exposure mechanisms to enable quinones, metals and other organic agents to produce toxicity. These models are outlined in Figure 5. In the first case, the organic compounds and metals become bioavailable through dissolution and cellular uptake, 2) macrophages and epithelial cells phagocytize particles and generate ROS and electrophilic chemistry in the intracellular milieu; 3) particles come in contact with cells followed by dissolution and uptake; and 4) particles are adsorbed onto cells with subsequent generation of

ROS and toxicity. In this latter case reducing equivalents are required to facilitate the process; elemental carbon may play a role as an electron transfer medium.

These hypotheses and mechanistic considerations translate into four related studies:

Evaluation of the airborne concentrations of different classes of organic compounds including quinones, nitroaromatics and carbonyl compounds in ultrafine, fine and coarse particles collected from diesel engines, freeways, source (source-site with no direct impact from freeways and no demonstrable products of atmospheric chemistry)/receptor (site where products of atmospheric chemistry are important) and Children's Health Study (CHS) sites (see below) in the Los Angeles Basin (LAB) to determine whether the agents are present in measurable concentrations, their distribution as a function of size, their temporal and spatial characteristics and their relation to toxicity and health effects.

Development of in vitro chemical assays to assess the reactivity of different PM samples with particular reference to generation of ROS.

Development and application of in vitro biological assays to quantitatively assess the toxicity of PM samples. Emphasis is on the use of yeast preparations, macrophages and epithelial cell lines.

The fourth study seeks to establish a murine asthma model which reflects the adjuvant effects of diesel exhaust particles (DEP) and concentrated airborne particulate (CAPS) in the lung and whose mode of action is consistent with injury by ROS and electrophilic agents.

Results

In vitro studies in macrophages revealed a range of biological responses in response to oxidative stress generation by DEP and CAPS. At low DEP extract concentrations, there is activation of the antioxidant element (ARE), which leads to the induction of a sensitive oxidative stress protein, heme oxygenase (HO-1). HO-1 is an antioxidant enzyme and is responsible for CO production in the exhaled air. CO is one of the most sensitive clinical markers for airway inflammation in human subjects exposed to DEP. At higher doses of DEP chemicals, macrophages and epithelial cells produce cytokines and chemokines. This likely forms the basis for the pro-inflammatory effects of DEP in the lung. These pro-inflammatory effects are mediated through activation of specific signaling pathways, including activation of the MAP kinase and the NF-?B cascades. Finally, extract doses >25 ? g/ml leads to cytotoxicity, which is mediated by a mitochondrial pathway. Epithelial cytotoxicity may contribute to airway hyperreactivity in asthmatics. Assays were used to assess the toxicity of concentrated coarse and (C) and fine plus ultrafine (F+UF) PM. F+UF particles induce oxidative stress (HO-1 expression) more readily than C particles. This effect correlates with a higher OC and PAH content of the F+UF particles. All considered, these findings suggest that the generation of oxidative stress leads to a stratified cellular response, which can vary from protective to pro-inflammatory or cytotoxic depending on the level of oxidative stress.

We experimented with a variety of murine exposure protocols to develop an asthma model. The hypothesis is that OVA by itself will lead to immune tolerance but co-administered DEP will induce allergic sensitization. To date, we achieved the best results with a 10-day inhalation

protocol in which aerosolized ovalbumin (OVA) and DEP was used. This model clearly demonstrated that DEP co-administration enhances OVA-specific IgE and IgG_1 production. This model was also used to show that interference in oxidative stress by thiol antioxidants will inhibit the adjuvant effect of DEP in IgE and IgG response induction. We have some preliminary success with a nasal challenge model, in which DEP is co-administered with OVA to induce allergic inflammation and airway eosinophilia in mice.

New assay procedures have been developed to expand the ability of the Center to evaluate air pollution samples for compounds of toxicological interest. We have developed a GC/MS procedure to determine the concentrations of selected quinones in air pollution particles. This procedure has been applied to diesel exhaust particles and air pollution samples. We have collected extensive data on quinones, and PAHs (Figures 6a-b). There are indications that napthoquinones decline in concentration across the LAB whereas phenanthroquinones are increased as a result of atmospheric chemistry. These results have toxicological significance when coupled with the results from our in vitro, in vivo, and human epidemiological studies.

We have also been studying the ability of quinones and diesel exhaust particles to catalyze the sulfhydryl mediated reduction of oxygen. This reaction, which generates superoxide, may be useful in predicting the potential toxicity of a sample by its ability to generate free radicals. The reaction will be used to compare small quantities of air pollution samples for their potential toxicity. The limited quantity available of air pollution samples and the need to compare them on a regional basis require a high throughput, highly sensitive procedure. We are currently studying yeast as the cellular probe because of its ease of manipulation and its ability to grow in the presence and absence of oxygen. Since the free radical based toxicity of air pollution is oxygen dependent, this form of toxicity can be evaluated in yeast by determining the oxygen dependent and independent toxicities.

Our research has demonstrated quinones, DEP, and PM are capable of generating ROS and we have established quantitative assays to compare toxicity as a function of size, composition and concentration. We have demonstrated reduction in toxicity when yeast are exposed to quinones, DEP and PM in the absence of oxygen suggesting that at least part of the inhibition of growth derives from ROS and yeast deficient in superoxide dismustase are dramatically impacted by quinones and DEP when the inhibition of growth is evaluated.

A related study to the four investigations described above is underway to evaluate particle-based hydrogen peroxide and organic peroxides concentrations. It will not be described in detail here. The study is complementary to the investigations emphasizing quinone toxicity and chemistry. It is being conducted by Dr. Suzanne Paulson of UCLA Department of Chemical Engineering. Field measurements of gas and aerosol phase hydrogen peroxide and organic peroxides are being conducted, the aerosol measurements for the first time. Summer ambient aerosol peroxide concentrations are 5-100 times the peroxide levels necessary to induce lung epithelial cell damage in lab studies. These studies will be expanded to investigate the generation of ROS producing organics and metals from particle bound peroxides and hydroxyl radical.

In the future, we will apply the murine asthma model to study CAPS effects. The goal of these studies is to establish an animal model that responds to organic DEP and CAPS components with

allergic airway inflammation. We will introduce the Buxco box in these studies to measure airway hyperreactivity (AHR). This will allow us to use intervention approaches, such as use of antioxidants, to interfere in the AHR. We will also test the effect of gene knockout in these animals to obtain an idea of the role of antioxidant and PAH detoxification pathways in the lung.

The *in vitro* toxicity studies will continue with CAPS collections by a liquid impinger to elucidate the toxicity of coarse, fine and ultrafine particles collected at freeways. We will also collect particles at various source/receptor sites to study the *in vitro* toxicity of organic compounds that are formed via atmospheric transformation in the LAB. We will continue to develop highly sensitive in vitro assays to test relevant mechanistic hypotheses and make use of genetically altered biological preparations to assess specific mechanistic features of the PM toxicity. We already have striking results with the use of yeast preparations deficient in superoxide dismustase (SOD).

Within the next three years, the analytical facility will provide comparative analytical data for PAHs, quinones and other relevant chemical species in air pollution samples from different regions of the LAB. We will provide analytical data on the redox activity of different samples to compare toxicity. Similarly we will provide toxicological data comparing samples from different regions of the LAB for free radical based toxicity and for oxygen independent toxicity. We will continue to develop highly sensitive in vitro assays to test relevant hypotheses and make use of genetically altered biological preparations to assess specific mechanistic features.

B. Studies of emission sources and adverse health effects

There are 4 specific studies underway under this general category:

1. **Freeway study**
2. **Traffic Density - human studies**
    a. **Children Health Study (CHS)**
    b. **The role of quinones, aldehydes, PAHs, and atmospheric transformation products on chronic health effects in children.**
    c. **Reproductive health**
3. **Particle characteristics-people are exposed to which particles from freeways?**
4. **Exposure and airshed modeling applications**

1. Freeway study - Hypotheses

- ?? **Mobile source emissions will exacerbate airway inflammation and allergic airway disease and produce cardiopulmonary effects;**
- ?? **The magnitude of allergic airway disease and cardiovascular effects from mobile sources are a function of the size distribution of PM;**
- ?? **Exposure in proximity to selected freeways with either heavy diesel or gasoline powered vehicles will cause exacerbation of inflammatory airway health effects and exposure to ultrafine particles at very close proximity to a freeway will result in the most severe effects**

These hypotheses have resulted in a series of near-source studies of concentrated fine and ultrafine ambient particles. A compromised (asthma) animal model is being used to study allergic airway disease associated with exposure to CAPs at varying distances from freeways as well as an upwind site. The study specifically seeks to determine the relative toxicity of PM in relation to particle size and composition including quinones, PAHs, and metals as the distance from the freeway varies.

We are now able to position mobile fine (F) and ultrafine (UF) concentrators at varying distances from a freeway with heavy diesel traffic to determine if exposure to emissions from the freeway demonstrates an adjuvant effect of CAPs in relation to asthma in animal models, and we are able to develop protocols which vary the concentration of particulate being studied in order to evaluate dose-response characteristics. The availability of both fine and ultrafine CAPs provides a basis to study the effects of UF particles and F+UF with implications for the role of fine particles as well. The study assesses exposure to traffic related pollution to further establish a causal link between asthma enhancement and adjuvant effects on asthma and mobile source emissions.

To test hypotheses that mobile source particles, especially those in the ultrafine size mode, would increase allergic reactions and exacerbate inflammatory responses in mice with allergic airway disease we exposed ovalbumin-sensitized and unsensitized mice to CAPs. The pattern of observed responses was consistent with the multi-phased mechanism for allergic response. The assays selected as markers of cell injury (total protein and beta glucuronidase) and inflammation (numbers of inflammatory cells) suggested even two weeks after the exposure, there was ongoing injury and inflammation in the lungs of CAPs exposed mice. The multi-phasic dose response mechanism was further elucidated by evaluating a series of doses that bracketed the ranges in which the allergic response, the anti-oxidant response and the toxic response occurred in our allergic mouse model. We found that exposures to CAPs at high concentrations resulted in reduced ability to respond to an allergen challenge, while exposures at lower concentrations evoked an allergic response (Figure 7). The mechanism(s) for reduced responses following high concentration exposures is unclear, but could include cytotoxicity to immunologically active cells or perhaps TH1/TH2 shifting. Using intermediate concentrations of CAPs, we have, clearly shown a pattern of allergic responses demonstrated by coherent increases in numbers of eosinophils and increased concentrations of OVA-specific IgE and IL-5 following exposures of mice to CAPs at a site 50 m downwind of a heavily trafficked freeway. We are currently performing exposures at increasing distances from the freeway.

The research described in **Section A of this report** is directly relevant to this study; airborne concentrations of quinones, PAHs, nitro-PAHs and metals are being determined to assess the relationship between their presence and concentration as a function of particle size and the resulting biological endpoints in the animal studies.

The specific aims for the three-year period covering the period of 2002-2004 include: Conduct near-source toxicological studies of concentrated F+UF and UF ambient particles. The proposed studies will also include exposure of sensitized mice upwind, downwind and at various distances from a freeway more heavily impacted by gasoline engine vehicles. We will also collect coarse,

F+UF particles for in vitro toxicity studies from sites downwind of a gasoline-impacted freeway (Freeway 405).

2. Traffic density and health outcomes

Two specific research studies are underway in the CHS to address the cited hypotheses with a third to follow next year.

2a. Children's Health Study/Exposure to vehicular pollutants/traffic density: respiratory health

2b. The role of quinones, aldehydes, PAHs, and atmospheric transformation products on chronic health effects in children.

2a. Children's Health Study

A series of related studies take advantage of the long-standing, important CHS. The CHS has demonstrated chronic respiratory effects in children exposed to air pollutants, and continues to explore findings from a decade of investigation. Mobile source emissions appear to have direct relevance to the findings of the CHS. The SCPCS has determined its greatest potential contribution to the CHS is through participation in traffic density studies and support for enhanced exposure assessment at road sites and also with emphasis on organic products of atmospheric transformation. In particular, hypothesis A has direct relevance to the traffic density studies insofar as it seeks to better define etiologic agents with relevance to chronic health effects identified in the CHS. We are studying the role of quinones and PAHs at CHS sites. The freeway study will have direct relevance to traffic density research described here. The source/receptor study (see below) will provide insights to the findings of the CHS. The following hypotheses guide the SCPCS-CHS epidemiologic studies.

- **Lung growth in children is permanently affected by air pollution in Southern California.**
- **Respiratory Illnesses are more frequent and severe in children living in areas of high traffic density.**
- **Chronic health effects in children can be attributed to a specific pollutant or combination of pollutants derived from mobile sources**

We are examining the relationship between traffic assignment and primary respiratory health outcomes in the CHS. We are examining the relationship between traffic at schools and homes and:

- The resultant exposure to traffic-related pollutants, such as PM_{10}, NO_2 and CO measured at schools and homes;
- the prevalence of asthma and wheeze at entry into the study and the incidence of asthma during follow-up; and
- the level and growth in lung function during follow-up.

?? In addition, we will examine how activity patterns such as time outside and mode of transportation to school, and polymorphisms of genes coding for enzymes involved in the metabolism of reactive species, modify the effect of traffic exposure.

We have geo-coded the home of each participant in the CHS and generated estimates of freeway related pollutants at these homes, using the CALINE4 model and measured and estimated traffic counts available from the California Transportation Authority in each of the CHS communities. This line source air quality model was developed as a planning tool to predict roadways which would exceed short-term CO standards, and we are adapting it to examine chronic health effects in the CHS. We have used CO as a surrogate for pollutants in fresh vehicular exhaust. Prevalent wind direction and speed is modeled in estimating home concentrations. Predicted CO from measured short-term traffic counts has been validated in other studies against short CO measurements, and our preliminary results suggest that modeled CO reflects measured long-term concentrations.

Using these models, we have conducted extensive analyses of the effect on respiratory symptoms at entry into the CHS cohort of estimated CO at children's homes. Our preliminary results suggest a large increased risk of parent-reported, physician-diagnosed asthma associated with exposure to local freeway derived CO. The effect of traffic was strongest for asthma diagnosed before age 6. In the CHS community with the highest estimated traffic exposures, there was almost a 10-fold increase in early onset asthma We have also found that modeled traffic-related pollutants are associated with lower lung function (FEV-1). Another pollutant heavily influenced by local traffic, NO2 at children's homes, was measured (not modeled) in a smaller data set, and a similar large association with asthma prevalence was observed. These results provide important new evidence linking asthma with air pollution.

We do not believe that CO is responsible for the observed effects, rather that it may serve as a surrogate for the pollutants of interest. Therefore, in the next three years we will improve estimates of vehicular pollutants from surrounding freeways and from surface streets in each community. We will estimate exposure at CHS homes and schools to NO2 and coarse and fine PM and elemental carbon, in addition to CO, using better models of emission factors recently available for California. Information on diurnal variability in traffic patterns, and in wind speed and direction and mixing levels, and the mix of diesel and light duty vehicles will improve these predicted pollutant concentrations at the times of day when children are exercising outside. We will validate these predictions against short term and long-term historical measured exposures available for these pollutants in the CHS communities. We will re-examine the effect on asthma and lung function of these improved estimates of traffic related particulate matter. The CHS also provides a rich data set of information on more than 350 incident cases of asthma identified during the course of the study, and the relationship between traffic exposure and incident asthma in these older children will be examined. A large portion of the participating children have been genotyped for polymorphisms in genes involved in the metabolism of reactive oxygen species, and we will examine how this genetic variation modifies the effect of traffic related particulate and related pollutants.

We plan more in-depth examination of the relationship of asthma with traffic related pollutants. These include a case control study of children in the CHS, which will measure traffic related

pollutants at the homes and schools of children with and without asthma. In addition, we are planning to identify and follow a new cohort of children, among whom we will carefully characterize new onset asthma, and for whom we will further examine the relationship of asthma to measured home traffic related pollutants, and to interactions of traffic related pollutants with activity patterns of individual children and with measured indoor allergens. These efforts have been made possible in large part by the productive collaborations facilitated by the Center. Findings from the clinical toxicology studies on gene expression will be applied to studies of free-living human populations exposed to contrasting PM levels. The models of traffic related pollution will be used to study additional health outcomes in planned studies.

2b. The role of quinones, aldehydes, PAHs, and atmospheric transformation products on chronic health effects in children.

This project is directly linked to the studies described in Section A. This study demonstrates one of the linkages between toxicological investigation and epidemiology.

To assess inter-community variability of vehicular-related emissions across Southern California, and to potentially link the variability of specific reactive compounds in ambient air to observed patterns of respiratory health in Southern California children, Center investigators have embarked upon an ambient PAH/carbonyls/quinones sampling study. Field operations are being conducted in twelve Southern California communities to collect 24hr samples, approximately once per week on differing days, to develop seasonal profiles of PAHs, aldehydes, and quinones for the twelve communities participating in the CHS. Center investigators expect that inter-community variability of specific organics, carbonyls, and quinones monitored in the study may help to disentangle previously observed correlated associations between gaseous entities, particle pollutants, and several respiratory health outcomes of California school children.

Samplers were deployed in Spring 2001 for actual data collection. Data available for the first sampling cycle (May-July 2001) suggest a regional transitional increase in quinones across the initial sampling sites, with a several-fold increase across communities in two of four quinones and virtually no change regionally in a third quinone (Figures 6a-b). Ambient napthalene concentrations varied from 86 to 650 nanograms per cubic meter, with Los Angeles basin sites having higher observed levels. Other semi-volatile PAHs varied in levels and order across the sites.

Field operations continued at Lompoc, Upland, and Mira Loma from August through October 2001; those data are currently being analyzed. During November and December 2001, sampling was conducted at the initial three sampling sites (Atascadero, San Dimas, and Riverside). In January 2002, samplers were once again re-located to Lompoc, Upland, and Mira Loma and will remain there for the January/February 2002 sampling period. Alternating two-month periods of sampling will continue between these two sets of three Southern California communities through May 2002. Sampling at the remaining six Children's Health Study sites is planned for mid-2002 through 2003. In this manner, we plan to develop seasonal information about inter-community PAH, carbonyls, and quinones in a cost-effective, logistically feasible, and scientifically credible protocol. The data will then be used in ongoing analyses of health outcomes for the CHS study

and toxicological endpoints of interest in animal studies planned for several locations across LAB.

2c. Reproductive outcomes

A third study involving Dr. Beate Ritz of UCLA and her colleagues on the relationship between traffic density and reproductive outcomes is also underway: "Residential Proximity to Traffic and Adverse Birth Outcomes in Los Angeles County, California, 1994-1996"

Conceptual hypothesis: Pregnant women who live in close proximity to traffic and are therefore exposed more heavily to primary motor vehicle emissions during pregnancy have a greater risk of delivering a low birth weight and/or premature infant.

Operational hypothesis: Pregnant women who lived close to heavy traffic roadways, including freeways and major arterials, in Los Angeles County, CA between 1994-1996 had a greater risk of delivering a low birth weight and/or preterm infant, taking into account season and ambient background air pollution levels and adjusting for individual and census-tract level risk factors for adverse birth outcomes.

This project is evaluating this hypothesis by observing the association between risk of LBW and preterm birth and residential proximity to heavy traffic roadways in women who lived within Los Angeles County between 1994-1996 (using addresses as stated on birth certificates) taking into account season and ambient background air pollution levels and adjusting for individual and census-tract level risk factors for adverse birth outcomes..

3. Particle characteristics-people are exposed to which particles from freeways?

Ultrafine PM consists of primary-source particles mostly emitted by combustion associated with motor vehicles. Because of their lack of mass, ultrafine particles have been impossible to collect in measurable amounts over practical time intervals. Also, the very small size of these particles has prevented determination of their size distribution. These two problems have been solved by implementing two technologies in series, an ultrafine concentrator and a recently developed cascade impactor, the NanoMOUDI. The combined concentrator/NanoMOUDI system has been employed in the field at two different locations in order to collect the ultrafine particles a function of morning (rush hour), afternoon (chemical reactions/agglomeration), and evening (rush hour). Results on the size distributions as well as the chemical constituents in each size range are unique.

We have been conducting a series of animal exposures to CAPs in discrete distances from a freeway as well as collecting size-fractionated PM for in vitro studies. We have measured the physicochemical and biological characteristics of PM as a function of distance from freeways. Specifically, the size distribution of PM was measured 10, 30, 50 100, 150, 500 and 1000 m downwind and 10m upwind the freeway. Elemental carbon, NOx and CO were measured in these locations. Finally the vertical profile of PM concentrations were measured. Our results defined a “zone of influence” of the freeway beyond which the number concentrations of PM decreases sharply. Concentrations of PM (number), EC, CO, and NOx were found to decrease

exponentially with distance from the freeway. The fastest decrease in concentration occurs for particle in the range of 5-20 nm, which coagulate rapidly to form larger particles. We have demonstrated particle coagulation occurs within the first 30-40 m from the freeway (Figure 8).

We will continue to study the following hypotheses in relation to ultrafine particles: 1) A physical model can be developed to predict the changes in size distribution and number concentration of ultrafine particles as they are carried away from a freeway by the wind; 2) Ambient ultrafine particle concentration adjacent to a freeway can be predicted from traffic density and speed together with meteorological factors.

Over the next three years we will conduct vertical profiles of ultrafine particle concentration and size distribution and horizontal profiles at locations near other freeways which have a high volume of diesel trucks. All these results will be used to develop a model that predicts exposure to ultrafine particles from freeways in terms of traffic density, topography, and meteorology.

4. Exposure and airshed modeling applications

The principal objective of the modeling component of the SCPCS is to develop more precise linkages between emission sources, human exposure, and health outcomes, including at the individual level for the CHS cohort and at the population scale for the LAB.

A principal hypothesis is that better characterization of the exposure of individual children in the CHS cohort will increase the robustness of observed health outcomes.

Related hypothesis: Within the framework of the coupled SMOG/REHEX modeling system, SCPCS field and laboratory measurements can be extrapolated to estimate the population-wide implications of specific airborne materials--gaseous and particulate--and to identify and quantify specific source contributions.

We are currently developing and applying models to support the existing research program of the SCPCS. This work utilizes UCLA's Regional Human Exposure Model (REHEX) and Surface Meteorology and Ozone Generation (SMOG) airshed model. The work has the following principal objectives:

- To provide modeling support for several of the projects currently underway in the SCPCS and the CHS—including the freeway and traffic density related studies, the mobile source emissions trajectory study, and the mobile source emissions exposure assessments.
- To provide a modeling component to the extensive pollutant measurement programs underway in both the Supersite and the various individual projects of the SCPCS/CHS.
- To offer a possible means for extrapolating the community or near-roadway pollutant levels associated with specific toxicological, animal and human health outcomes to susceptible or most heavily impacted sub-populations, as well as to the entire regional population of southern California.
- To create linkages between the science emerging from the SCPCS/CHS programs and the policy and standard-setting goals established by the NRC and EPA.

?? To aid in the design of cost-effective and optimally health-protective emission control strategies for fine particles and particle-associated species.

Progress was made this year in modeling the distributions of particle-borne materials and gaseous pollutants across the LAB. These results represent the initial applications of the SMOG model to characterize agents of interest to the SCPCS health research team. The distributions of PAHs such as naphthalene, and the formation of secondary organic compounds like the quinones and nitro-PAHs, are largely uncharacterized in the LAB. Some of these primary and secondary organic species—both in vapor and particulate phases—represent potential health hazards, and therefore provide a focus for the SCPCS health studies. In most cases, the species abundances, as well as those of numerous co-generated pollutants, are rarely measured except in local intensive field campaigns, and for limited sampling periods. Many potentially important compounds, especially organic radicals, peroxides, acids, and quinones are not usually detected owing to difficulties with instrumental sensitivity and specificity, although such compounds are likely to be relevant to health effects research. Therefore, we have applied the SMOG model to carry out simulations that depict the distributions of key organic species. It is noteworthy that these simulations also yield the distributions of a wide range of common co-toxics and criteria pollutants, both gas and particle. In our initial simulations, the sources, dispersion, and photochemical decomposition of naphthalene, and the production and disposition of naphthoquinones, were considered. The key reaction in the decomposition of naphthalene involves OH addition, for which the rate constant is known. Naphthalene also reacts with nitrate radicals, although this path is more effective at night. In the presence of NO, the yield of naphthoquinones and related species via the OH reaction can be as large as ~40%, depending on the number of compounds counted in this reactive cohort. The direct yield of 1,4-naphthoquinone is roughly 1%. As a result, the distributions of naphthalene and its principal secondary quinone products can be reasonably estimated.

Figure 9 illustrates the simulated distribution of naphthalene across the LAB, averaged over a typical summer day with 1998 emission rates. Naphthalene emissions are associated with fuel vaporization, and predicted distributions correlate weakly with transportation corridors. Clearly, atmospheric dispersion is a critical factor in controlling the regional distribution). Fuel refining is a major source of naphthalene in the western coastal area (Figure 9). Dispersion creates a plume that extends as far as the Banning pass in the eastern basin.

The corresponding distribution of the 1,4-naphthoquinone byproduct of naphthalene oxidation is shown in Figure 10. Striking differences are seen between the concentration patterns of the primary and secondary compounds—naphthalene versus naphthoquinone. The 1,4 naphthoquinone is concentrated inland along mountain slopes where air trajectories arrive from the coastal region. While naphthalene is partitioned mainly into the gas phase, 1,4 naphthoquinone has a substantial particulate component as well. The partitioning of the quinone explains to a large extent the higher predicted values compared to measurements in Figure 8; the model yields the total 1,4 naphthoquinone abundance, including that in the vapor and on all particle sizes, whereas the data represents only the portion on $PM_{2.5}$. Once the SMOG model is fully calibrated, comparisons between model simulations and field observations can be used to obtain better estimates of the actual vapor/particulate partitioning of organic compounds in ambient air.

We have not yet utilized field data collected during the SCPCS aerosol characterization studies to calibrate sources of naphthalene and other organic species. This will be done in the near future to confirm and/or adjust emission inventories.

Finally, we are pursuing the coupling of the SMOG and REHEX models. The coupled modeling system will be employed to estimate exposures in designated CHS study areas, focusing on the school-age cohorts. This effort will provide an area-weighted evaluation of simultaneous exposure to a variety of agents, and will complement the higher resolution REHEX assessments based on census tract data. The well-characterized exposures from SCPCS freeway projects can then be extrapolated to these populations. At a minimum, we can identify which sub-populations and demographic groups experience levels of fine and ultra-fine particles, as well as selected particle associated species such as oxygenated compounds, which occur in the community or near-roadway environments for which health outcomes are observed by SCPCS investigators as the result of concentrating such pollutant levels using the Center's concentrator technology.

Use of the combined exposure/airshed models can also provide an ability to generalize to the entire regional population, findings from the SCPCS projects concerning the relative importance of primary vehicle emissions versus the role of atmospheric chemistry. The seasonal and source-receptor aspects of these studies are readily addressed by the SMOG airshed model, whose outputs can then be used as inputs to REHEX for estimating exposure and dose.

C. Effects of varying spatial and temporal patterns of ambient PM and co-pollutants and resulting health effects; particular emphasis on the role of atmospheric chemistry

1. Hypothesis – Source/Receptor study

- **Atmospheric chemistry is important in the toxicity of PM and co-pollutants, airway injury and cardiovascular effects will be greater at receptor sites downwind of source sites along the mobile source trajectory in the Los Angeles basin;**

- **Airway disease and cardiovascular effects will be more severe during periods of high photochemical activity in the summer than in periods of low photochemical activity during the winter**

To test these hypotheses we have installed ultrafine, fine and coarse particle concentrators and inhalation exposure systems in an existing trailer for animal studies and, after suitable evaluation and testing, use that facility to perform animal studies at a source (USC main campus in downtown Los Angeles) and two distinctly different receptor sites (Azusa and Upland). Receptor sites are those locations in which the PM composition has been modified by atmospheric chemistry with emphasis on hydroxyl radical and nitrate chemistry.

The rationale used for choosing the specific sites in the LAB for the source/receptor study derives from recent data. The data indicate that there are two distinct air trajectories in the LA Basin that transport the air pollutants emitted from the southwestern part of the LAB eastwards. There is a "vehicular emissions" trajectory that is tangential to freeways 10 and 210, which starts approximately in downtown Los Angeles and follows the direction along freeways 10 and 210.

Upland is a site adjacent to the U.S. 10 freeway and is along a trajectory primarily impacted by mobile source emissions. Azusa is also situated along this trajectory, but is intermediate between the sources (in downtown LA) and the "receptor" site, Upland, which is farther eastwards along this trajectory. In addition to the "vehicle-oriented" trajectory, there is a distinct wind trajectory that is called "Nitrate-Oriented Trajectory". This trajectory starts in the Long Beach area, where a significant portion of PM is mostly generated by non-mobile sources such as petroleum refineries, numerous energy generating facilities and heavy industry emission (although there is significant mobile source contribution). This trajectory progresses through areas that are upwind of, within, and downwind of the Chino dairy farms area, a large source of gas-phase ammonia. Because the main theme of the SCPCS is PM and compounds emitted from mobile sources, conducting studies along the "nitrate-oriented" trajectory, while of great scientific value, might dilute the focus on our research hypotheses linking health effects to vehicle-emitted pollutants, given the contributions of non-vehicular sources to PM found along that trajectory. Our plan for the next 3 years is to therefore perform health effect studies at each of the 3 sites along the "vehicle-oriented" trajectory during periods of high and low photochemical intensity, and to coordinate those studies with PIU sampling campaigns to maximize the degree of integration of our Center's efforts.

We have investigated the physicochemical characteristics of ultrafine particles in urban area both at source site and receptor site located Los Angeles Air Basin. From the semi-continuous measurement of size distribution of ambient aerosols as well as time-integrated sampling of particles, the temporal and diurnal trends in the behavior of ultrafine particles were conducted to better understand the formation mechanisms in different locations of an urban metropolitan area. Results showed that a western site, located in central Los Angeles, is appropriately characterized as a source site with constant primary emissions. In contrast, ultrafine and accumulation modes PM in a receptor site were found to originate mostly from secondary reactions, which are more pronounced during the warmer months of the year particularly during the warmer season of the year, between April through October.

The study will use two animal models, both of which have existing allergic airway disease (asthma). The models will be the OVA sensitized Balb/c mice and Brown Norway (BN) rats and in vitro studies described earlier will also be conducted. In both the in vivo and in vitro studies the mobile particle concentrators will be used to generate PM of known size ranges.

In the proposed project, Brown Norway (BN) rats with and without allergic airway disease will be concurrently exposed to either concentrated ambient coarse, F+UF or UF particles taken from the ambient air in three different locations in the LAB over a period of three years. In each location, one series of exposures will be conducted during a period of intense photochemical activity, in early October, and another series will be conducted in January, during which photochemical reactions are less intense. We have selected the BN rat treated with OVA as animal model of allergic airway disease because this rodent model has been used extensively in the laboratory of Dr. Jack Harkema of Michigan State University who will be our primary collaborator in this study and in other laboratories and is well characterized. It is one of the few animal models that has been used successfully to demonstrate that inhaled pollutants can enhance allergic airway disease. In addition, the rat provides us more pulmonary tissues,

compared to the mouse, to analyze using various microscopic, molecular and biochemical analyses as outlined in our proposal.

The OVA-sensitized mouse and BN rat models address two separate issues: The mouse model will be used to test the hypothesis that ambient particles can act as an adjuvant and will contribute to the development of allergic and asthmatic responses. The rat model tests hypotheses related to the exacerbation of asthma. Thus symptoms such as, airway inflammation, increased mucus secretion and increased storage of mucoid substances in lung tissue will be evaluated with the latter model. Results to date indicate that we are able to produce inflammatory responses in sensitized animals at the receptor site.

This study is closely related to the investigations described in **Section A** since we will characterize PM and vapor co-pollutants to determine the concentrations of quinones, PAHs, nitro-PAHs, and metals. We hypothesize the concentration of quinones and other oxygenated PAHs and nitro PAHs will increase across the LAB as a result of atmospheric chemistry. We shall investigate how degrees of toxicity vary in animal and in vitro studies as a function of size distribution and the composition.

2. Hypothesis – Human Clinical studies and CAPs

?? **Exposure to concentrated airborne particulate (CAPs) will affect cardiopulmonary health in normal persons and individuals with asthma, and chronic obstructive pulmonary disease (COPD).**

Within the context of this hypothesis, acute cardiopulmonary responses to fine, ultrafine and coarse CAPs in healthy human volunteers, and patients with COPD and asthma are being studied in a site on the western side of the LAB in reasonable proximity to multiple arteries but not within 500 m of a freeway. This site is considered a "source" site as a result of a lack of photochemical processes. Later, similar studies will be conducted in a receptor site to gauge the effects of atmospheric chemistry on cardiopulmonary responses in human volunteers.

This research makes use of the newly constructed concentrators to study the cardiopulmonary effects from exposure to CAPs. We have established a critical mass of technical and clinical experience, and lab facilities, and personnel to conduct controlled human exposure studies with concentrated ambient particles and co-pollutants using Center and other support. We have completed exposure studies of healthy, asthmatic, and COPD volunteers exposed to concentrated LA source-area ambient fine PM at worst-case concentrations. We have initiated exposure studies with concentrated fine PM and co-pollutant (NO_2) in healthy elderly and COPD volunteers with separate EPA funding. We have developed technology to extend the above studies to coarse and ultrafine particles. Multiple biochemical and heart-rate-variability endpoints are being analyzed.

Results are now available for healthy and asthmatic subjects exposed to concentrated fine particles (2 hours at approximately 200 micrograms per cubic meter). No pulmonary dysfunction was found, although there was a shift in airway cell populations recoverble by sputum induction (not obviously of an inflammatory nature). Slight changes in heart rate variability were found,

suggesting decreased sympathetic relative to parasympathetic influence on the heart. In addition, very slight but statistically significant increases in cardiovascular symptoms were found during/after exposure, while both heart rate and minute ventilation rate declined during concentrated particle exposure, relative to the filtered-air control condition. Finally, some blood biochemical indices changed in a manner to suggest increased coagulability after concentrated particle exposure. The major collaboration with the US EPA in the evaluation and comparison of human biomarkers of PM exposure and effects, e.g., in cardiac electrophysiology, blood and sputum assays will be continued and include intensive statistical analysis of pooled volunteer groups exposed to source-area fine PM with and without co-pollutants; test concentration-response relationships, implementation of new noninvasive measurements of lower-respiratory inflammatory response to particle exposure by expired gas analysis, extension of human exposure studies to fine and ultrafine particles.

We will conduct human clinical studies at receptor sites, using subject group(s) and size range(s) most likely to show effects. We will relate clinical responses of human volunteers to PM exposures and their genotypic profiles (with gene arrays) to characterize gene-environment interactions.

Through collaboration with other PM Centers we intend to conduct a Meta-analysis of human PM exposure findings here and elsewhere, to increase power to detect subtle effects. We shall conduct small-panel studies of individuals in source and receptor areas identified as "high risk" by earlier animal and human studies, using any noninvasive measures of response found to be sensitive in earlier controlled exposure studies.

SCPCS will apply the research conducted during years 3-5 on gene array techniques to relate clinical responses of human volunteers to PM exposures and their genotypic profiles to characterize gene-environment interactions.

III. Key Investigators and their Expertise (Figure 2 and 11)

IV. Non-research accomplishments-conferences, new collaborations, and other miscellaneous activities

SCPCS continues to hold monthly Executive Committee meetings to address Center related issues and to track progress of individual research projects. Internal advisory committee meetings also track progress and make decisions on whether adequate progress is being made in relation to budget allocations. The Director, Deputy Directors and Internal Advisory Committee make budgetary allocation decisions on an annual basis based on interim progress presentations and annual reports.

February 7-8, 2000, a major conference was held at UCLA entitled “PM Toxicity Studies: State of the Art and Future Directions“. This conference involved participants from the SCPCS, other PM Centers, U.S. EPA, and Local and State Air Agencies.

June 12-13, 2001, SCPCS sponsored a workshop with the Office of Environmental Health Hazard Assessment (CAL/EPA) entitled “Issues in the Assessment of Health Impacts of

Gasoline Emissions in California". Participants were from throughout the U.S., and included representatives from industry, HEI, U.S. EPA, Local and State Air Agencies, CAL/EPA, a wide range of universities and research centers including SCPCS.

An SCPCS dosimetry workshop to identify dosimetric issues relevant to our ongoing investigations was held this fall. Participants included faculty, postdoctoral fellows, students and staff. The Center has established a working group to investigate dosimetric issues associated with the concentration of organic contaminants, metals, and other species on PM. That working group has evaluated the relationship between the airborne concentrations of organic matter found on PM and the sensitivity of our in vitro assays to determine whether the assays require large amounts of material thereby calling the relevance of the determinations into question. Our theoretical evaluation confirms that the in vitro assays are being conducted in a dose range consistent with airborne exposures.

New research collaborations have been established with Dr. Kent Pinkerton of the University of California at Davis, Dr. Robert Devlin, EPA and Dr. Jack Harkema of Michigan State University. Dr. Harkema has been awarded a STAR grant to conduct studies with SCPCS faculty in the LAB over the next three years using our mobile ultrafine, fine and coarse concentrators. Dr. Jon Fukuto (toxicology/UCLA) and Dr. Robert Schiestl (environmental genomics/UCLA) have joined the SCPCS as investigators in the mechanistic aspects of PM toxicity. Dr. Beate Ritz (epidemiology/UCLA) has joined the Center in the area of reproductive outcomes and PM exposure. Dr. Suzanne Paulson (chemical engineering/UCLA) has also affiliated with the Center and has begun studies on hydrogen/organic peroxides in PM. Dr. Frank Gilliland (epidemiology, USC) has joined the SCPCS to develop research on gene arrays and human clinical studies.

Related grants have been successfully awarded for exposure characterization in the LAB. One contract awarded by CARB is designed to characterize children's exposure to diesel and other particles while commuting to school by bus. A second contract was also awarded by CARB; it evaluates exposures in urban classrooms selected for environmental justice concerns. These grants are only possible because of the SCPCS infrastructure including sampling capability and analytic chemistry.

The SCPCS has further solidified its relationship with CARB who has provided 5 year funding for construction, validation and application of mobile ultrafine, fine and coarse particle concentrators. On June 14, 2001 an all day meeting was held with representatives from CARB and the SCAQMD to discuss ongoing interaction in research. SCAQMD has been central to site location for our trailers for field studies in the LAB.

Seminars on various PM research topics are held monthly with the SCPCS Executive Committee meeting. A recent afternoon session was devoted to presentations by postdoctoral fellows and students on their research. A quarterly newsletter and website have been established.

V. Additional research ongoing or in development

Characterization of the airborne concentrations, size distribution, and other chemical/physical elements of PM within the LAB in relation to health studies.

There is fundamental lack of information on the size and chemical composition of ultrafine, fine and coarse PM, which are essential in understanding their formation mechanisms in different areas. The development of new sampling technology makes our Center uniquely capable of creating a database in various locations of the LAB. Direct emissions from vehicles and power plants have been considered the main sources of these particles. As our preliminary studies illustrated, this is a common misconception. Our ability to generate short time-scale data on size-fractionated chemical composition of ultrafine PM will help us understand how their formation processes, which in addition to direct emissions include vapor condensation, and secondary formation via photochemical reactions, depending on location, time of the day and season. The data on ultrafine PM will be of paramount importance in helping us understand the sources of these particles and ultimately implement effective control strategies.

We will continue over the next 3 years to collect size-fractionated PM at source and receptor sites in the LAB. This collection will be conducted in the same sites and time periods where animal inhalation studies/in vitro assays to CAPs are underway. We will continue the characterization of PM as part of our Supersite program (See appendix 1).

Gene expression patterns in susceptible humans exposed to concentrated ambient air pollution particles

The long-term objective of this program of research is to understand the mechanism for increased cardiopulmonary mortality associated with small changes in exposure to particulate air pollution. To accomplish this objective we are beginning to develop and test a sensitive method using gene expression microarrays to identify changes in gene expression in peripheral blood cells to be used in clinical toxicology studies of particulate air pollution. The general hypotheses of this study are: 1) acute exposure to concentrated ambient $PM_{2.5}$ in the LAB causes acute cardiopulmonary dysfunction in adult human volunteers; 2) the PM-induced cardiopulmonary dysfunction is mediated by local (airways) and general (systemic) inflammation and/or alterations in homeostasis; and 3) the above responses differ according to the health status of the subjects and/or the physical or chemical composition of the inhaled particles. The use of gene expression microarrays to assess temporal patterns of gene expression after controlled exposure to PM may provide further insights into the mode of action of PM in people with different health status and identify causal components in the complex PM mixture.

We will establish our collection, fixation, and separation protocols, and then assess intra- and inter-individual variability in gene expression to provide key information for study design and sample size estimates. Once methods have been developed, this innovative technology will be integrated into the ongoing clinical toxicology studies of the effects of concentrated ambient air pollution particles on adults with asthma and COPD.

VI. Major accomplishments and conclusions

One of the unique challenges and opportunities to this Center was to define research which took advantage of the geography of the LAB with its enormous air pollution problem. The size of the LAB and its complexity represented a key feature in our planning the research program. The challenge of the LAB itself was magnified by the opportunities and difficulties presented by bringing together outstanding scientists from 5 institutions in the Basin as well as investigators from UC Davis and Michigan State University and the need to interact with the California Air Resources Board and the South Coast Air Quality Management District. The third challenge was to bring together the different disciplines of epidemiology, toxicology, exposure assessment, aerosol science, dosimetry, and atmospheric modeling into an integrated, reinforcing series of activities. These three challenges would not have been addressed were it not for the existence of the Center. It is absolutely crucial to have an air pollution center in Southern California to be able to address the problems and opportunities of air pollution and research in the LAB.

During the first two and ½ years the SCPCS has been highly successful in launching multidisciplinary research that is 1) hypothesis based; 2) integrates into a central theme the various disciplines available in universities in the region; 3) seeks to address EPA needs in term of the Agencies long term research and regulatory objectives; 4) is closely linked with research underway in the other PM Centers; and 5) has produced highly relevant results consistent with the NRC research priorities.

The key accomplishment has been the ability to bring together the various disciplines into an integrated, interactive research activity in which the specific projects are linked by common hypotheses. The following provides a clear example of the integrated, focused nature of our research.

?? **Organic constituents associated with PM, including quinones, other organic compounds (PAHs, nitro-PAHs, and aldehydes/ketones) and metals, are capable of generating reactive oxygen species (ROS) and acting as electrophilic agents. They have a central role in allergic airway disease including asthma and cardiovascular effects through their ability to generate oxidative stress, inflammation and immunomodulating effects in the lungs and airways.**

In order to test this hypothesis we have developed 1) exposure assessment research designed to quantitatively characterize the organic constituents found on PM produced from sources (diesel vehicles, freeways with heavy diesel or gasoline), or produced by atmospheric chemistry; 2) in vitro toxicology studies designed to test the mechanistic hypothesis and to define the specific pathways responsible for health effects; 3) animal studies designed to develop dose-response relationships and test hypotheses linked with the exposure assessments; 4) conduct epidemiological studies linked directly with traffic density but also the quantitative determinations of relevant organics under 1); 5) carry out human clinical studies making use of the CAPs system and 6) conduct modeling studies to evaluate the distribution of the organics and metals across the LAB.

The quantitative exposure information coupled with in vitro and in vivo studies and epidemiological investigations and modeling studies suggest the hypothesis cited above has clear merit and represents a fundamental issue for further investigation. The success of the SCPCS research has also resulted in a clear commitment on the part of other PM Centers to conduct research based on the same hypothesis and to integrate activities across Centers.

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Figure 1

Central Theme of the Southern California Particle Center and Supersite =

PM From Mobile Sources

Three Different PM Exposure (or Air Pollution) Regimes

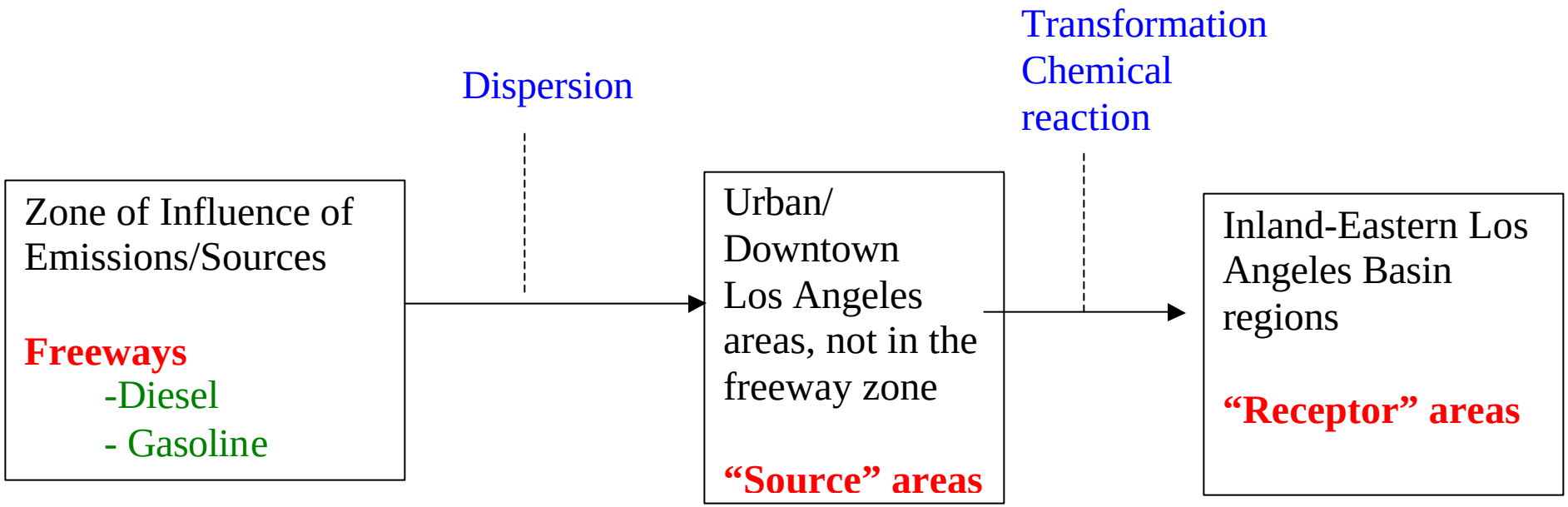

Figure 2

PM from Mobile Sources is the Central Theme of the SCPCS

Our Goal: Evaluation of how the effects of chemical and physical differences in PM of these 3 exposure regimes mediate biological responses

For each air pollution regime:

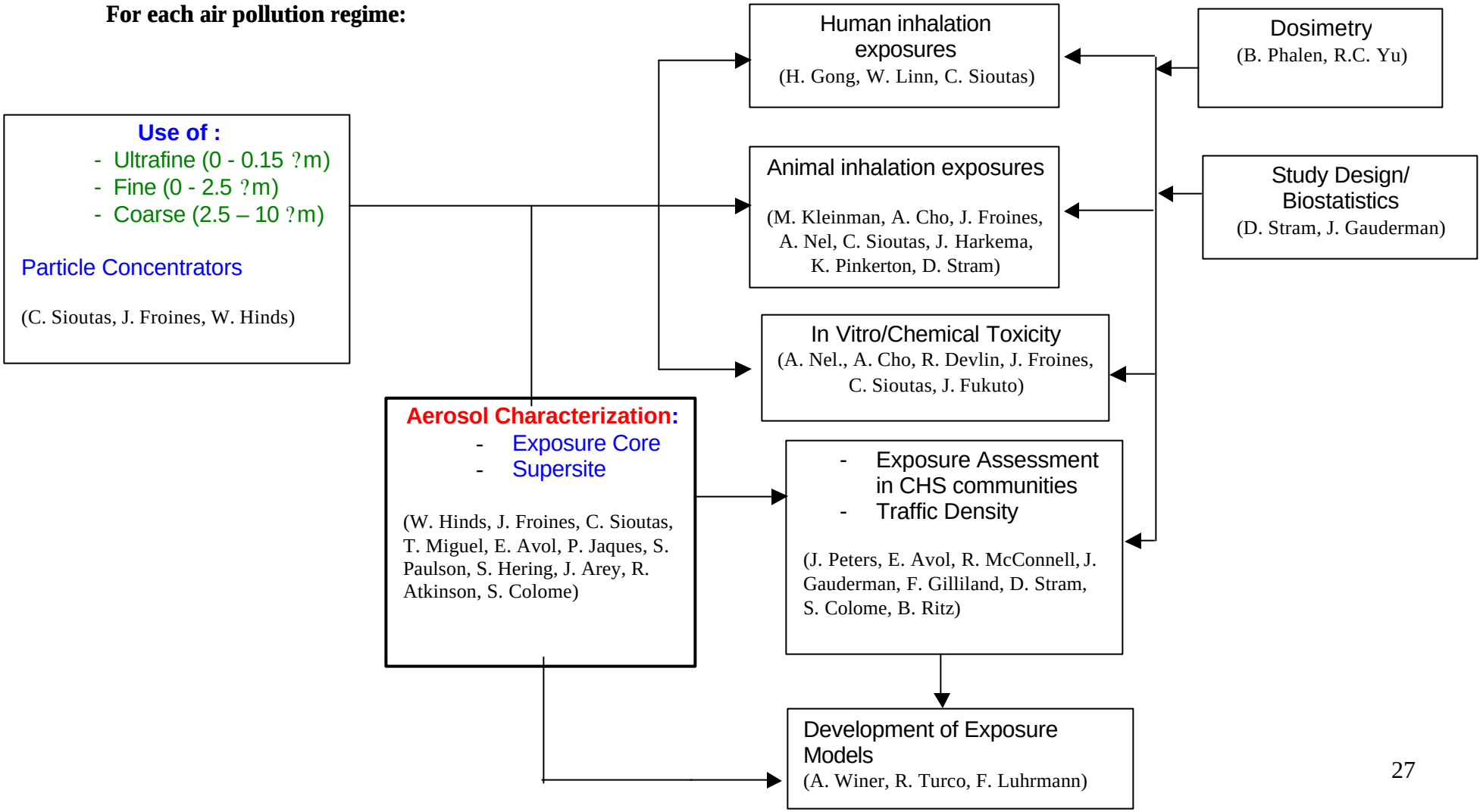

Figure 3

USC DW-Downey BH-Boyle Heights SD-San Dimas UP-Upland RV-Riverside

Figure 4

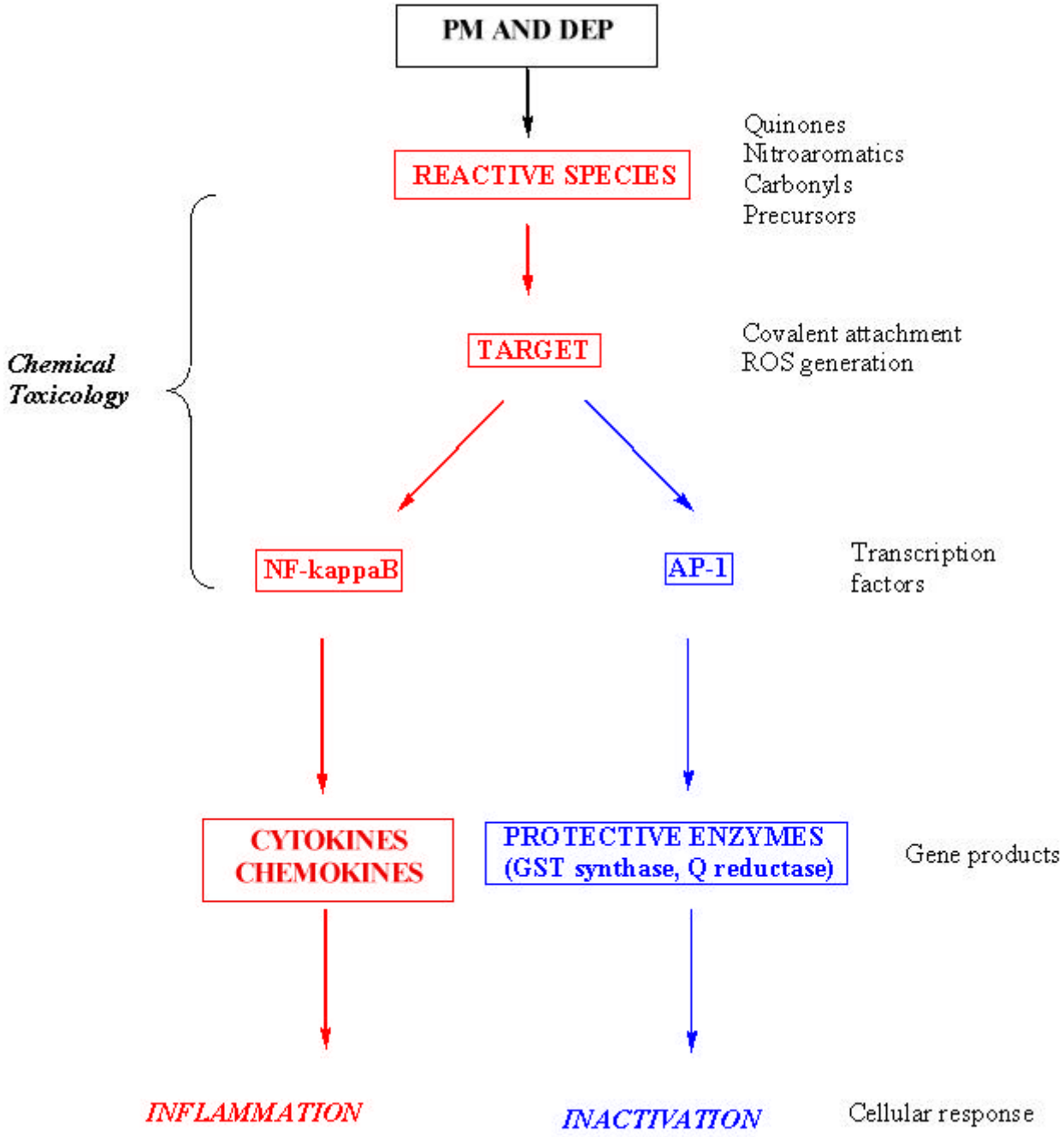
PM AND DEP
REACTIVE SPECIES
Quinones
Nitroaromatics
Carbonyls
Precursors
TARGET
Covalent attachment
ROS generation
Chemical
Toxicology
NF-kappaB
AP-1
Transcription
factors
CYTOKINES
CHEMOKINES
PROTECTIVE ENZYMES
(GST synthase, Q reductase)
Gene products
INFLAMMATION
INACTIVATION
Cellular response

Figure 5 Exposure Models

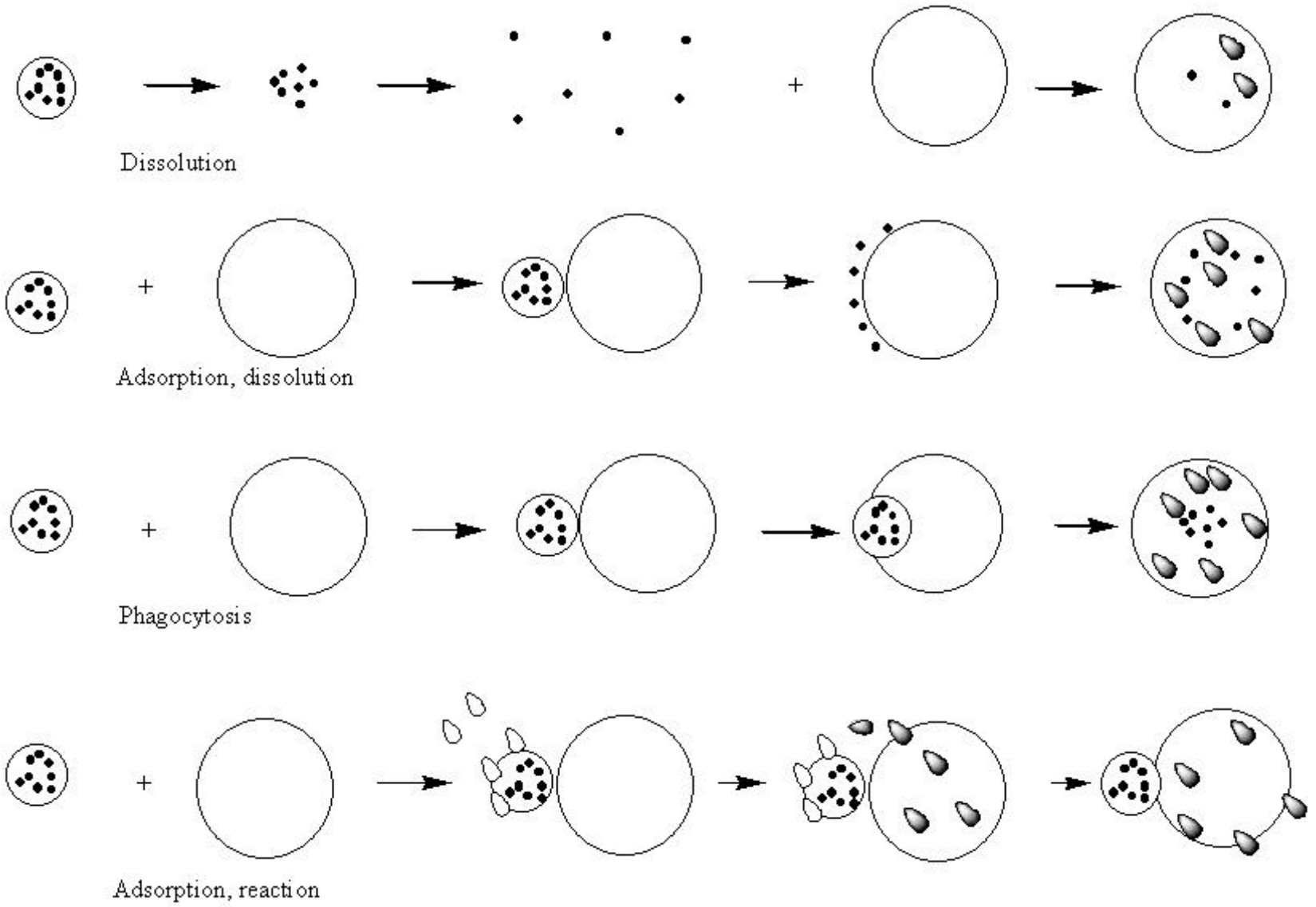

Figure 6a

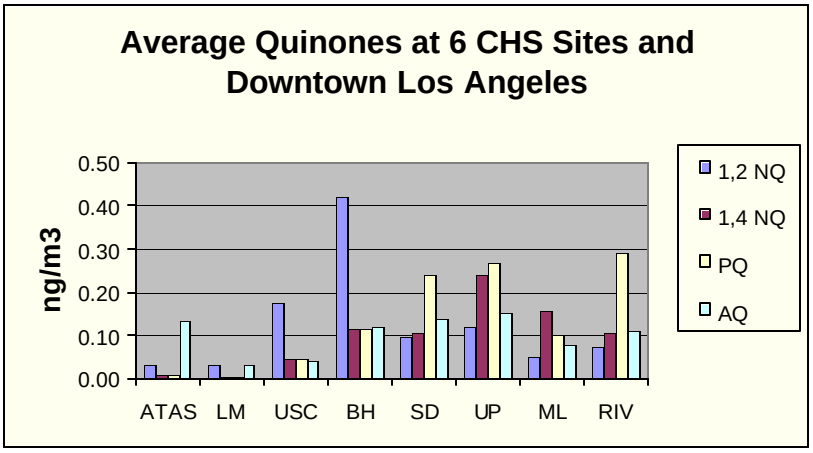

Figure 6b

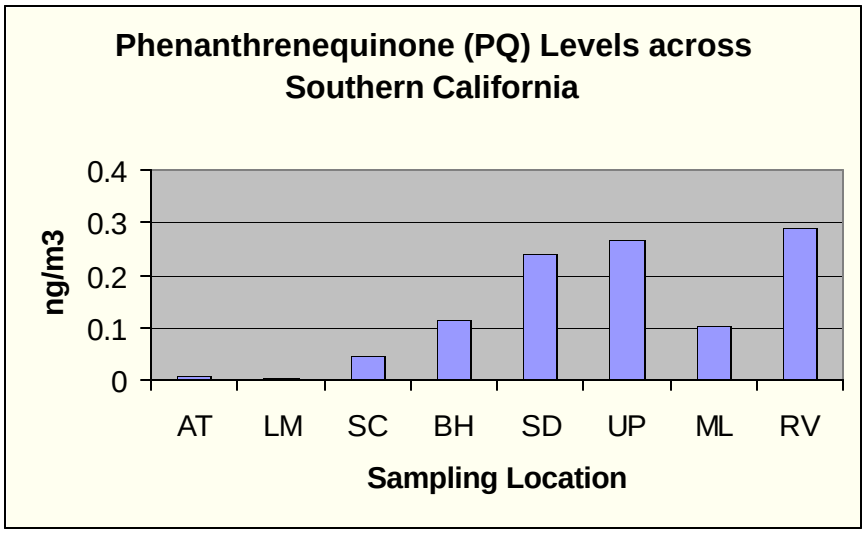

Figure Legend

ATAS(AT)	Atascadero (source site)
LM	Lompoc (source site)
SC	University of Southern California (source site)
BH	Boyle Heights (source site) – freeway impacted
SD	San Dimas (receptor site)
UP	Upland (receptor site)
ML	Mira Loma (receptor site)
RV	Riverside (receptor site)

Source site – site where little atmospheric chemistry is anticipated
Receptor site – site on eastern side of Los Angeles Basin where chemistry is expected

Figure 7

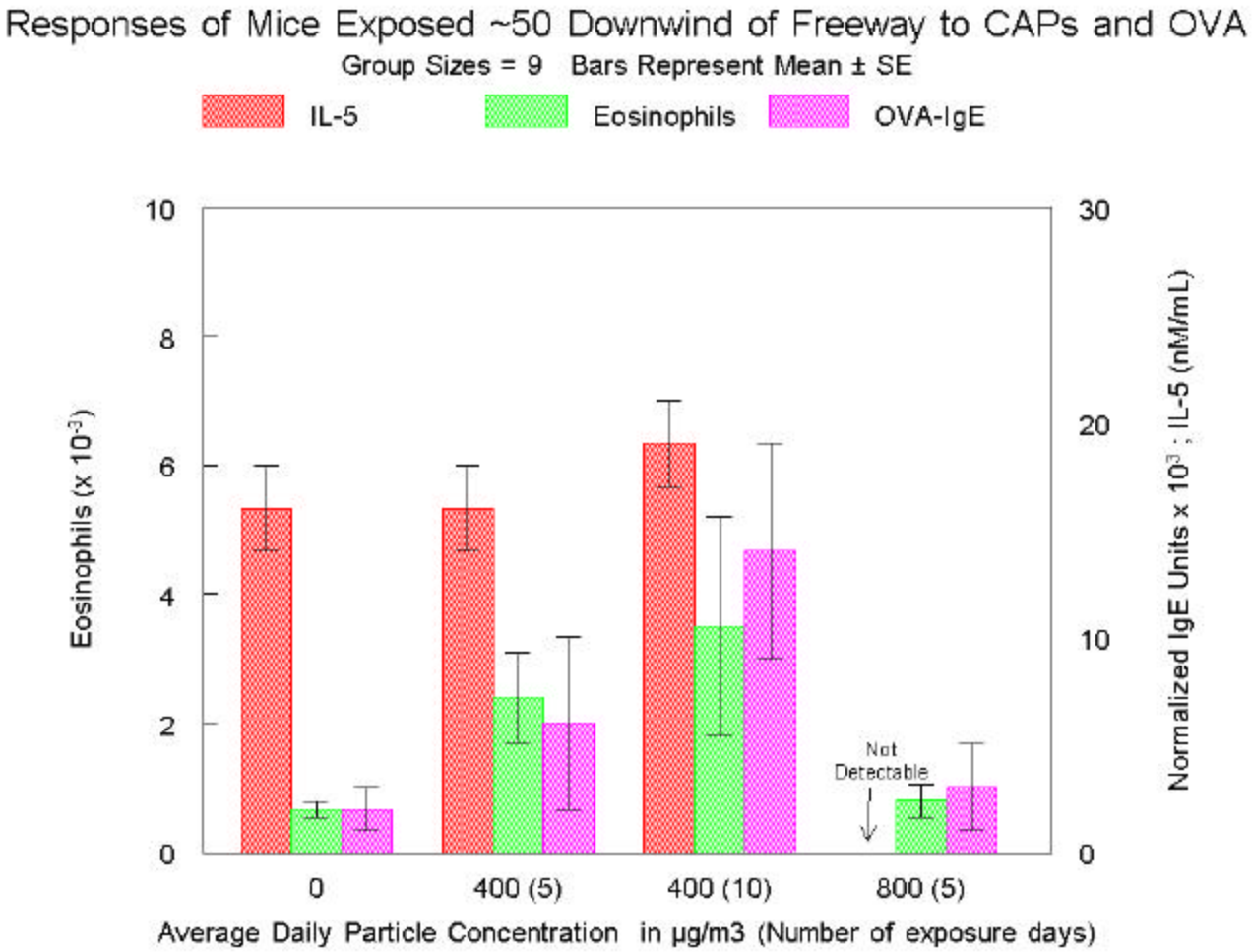
Responses of Mice Exposed ~50 Downwind of Freeway to CAPs and OVA
Group Sizes = 9 Bars Represent Mean ± SE
IL-5
Eosinophils
OVA-IgE
Eosinophils (x 10-3)
Normalized IgE Units x 10-3 ; IL-5 (nM/mL)
10
8
6
4
2
0
30
20
10
0
Not Detectable
0
400 (5)
400 (10)
800 (5)
Average Daily Particle Concentration in µg/m3 (Number of exposure days)

Figure 8

Relative Particle Number, Mass, Black Carbon, CO Concentration versus Downwind Distance from Freeway 405.

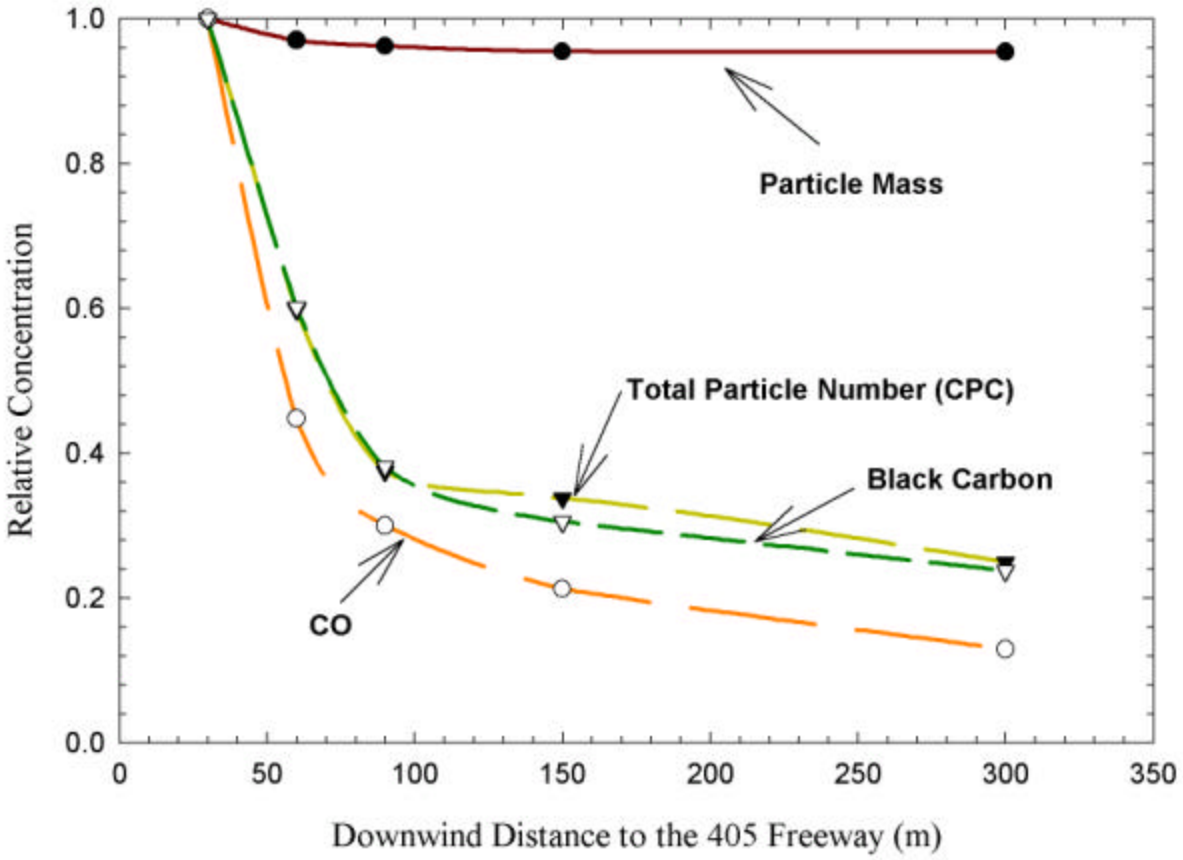

Figure 9

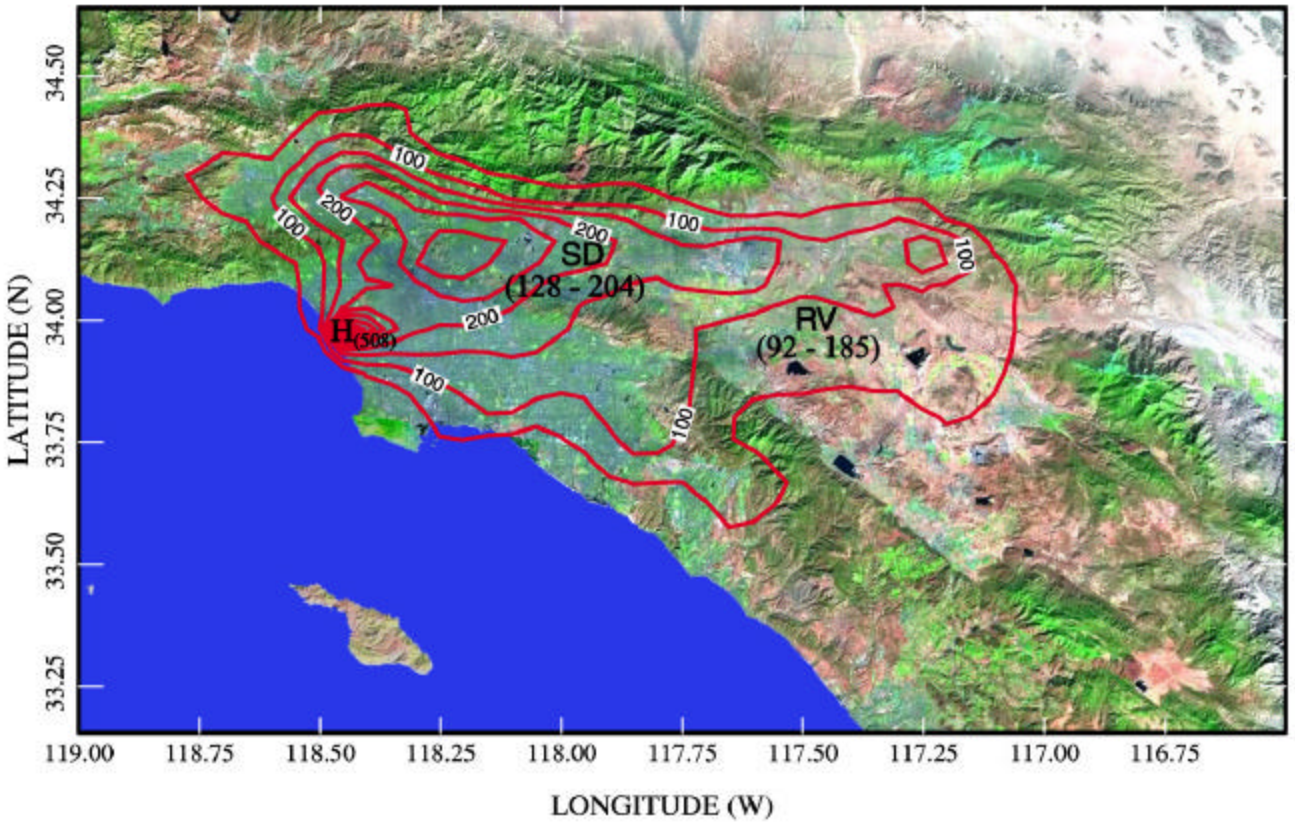

Figure 9. Predicted distributions of naphthalene across the Los Angeles basin corresponding to 1998 summer emissions (the contours are marked in units of ng/m^3). For comparison, SCPCS field data from San Dimas (SD) and Riverside (RV) are shown in brackets (the range of measurements over several days are given in the same units). The 24-hour averaged distributions indicate "hotspots" around refineries and freeway corridors. The emissions used in these simulations have not been calibrated against observations.

Figure 10

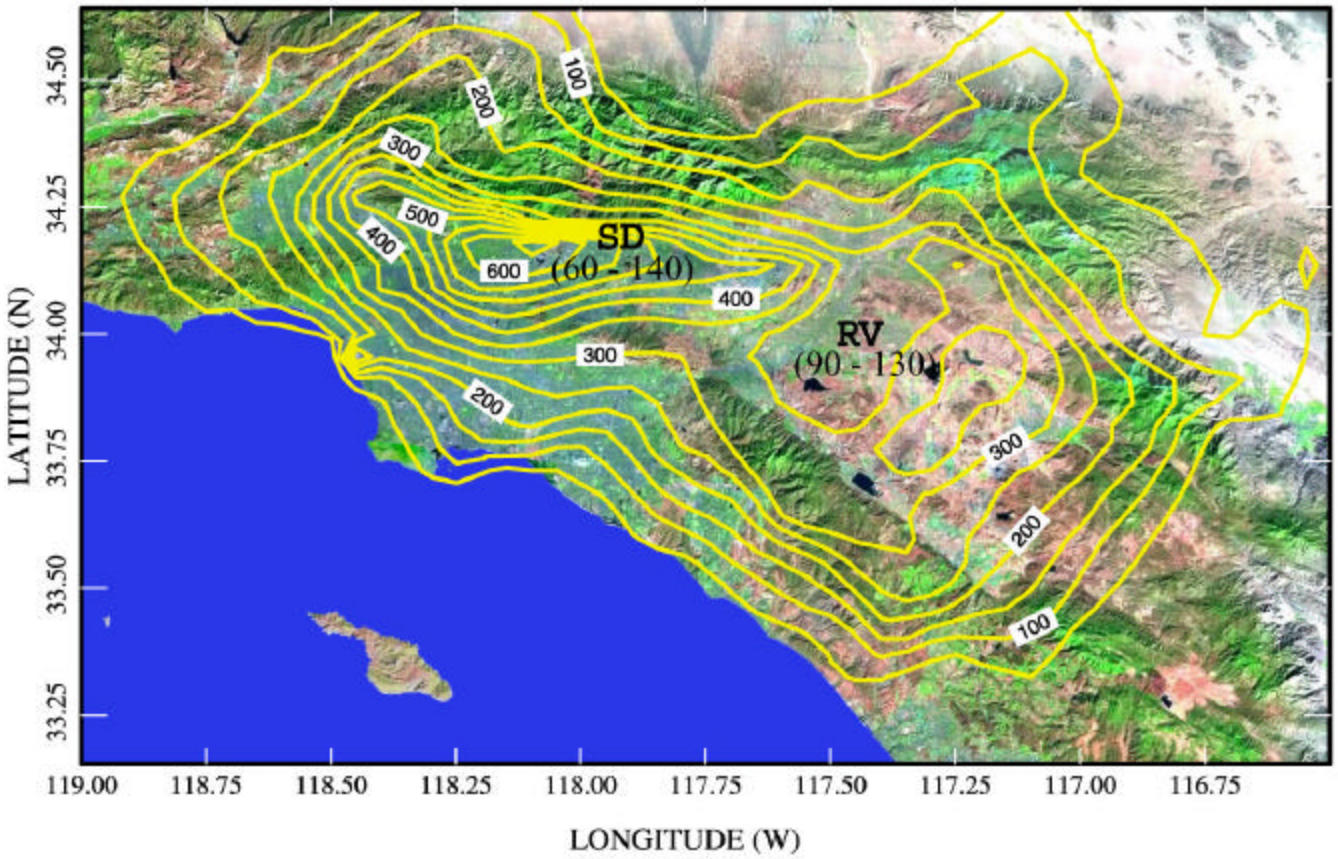

Figure 10. Shown are concentration contours for the 1,4 naphthoquinone byproduct of naphthalene (in units of picograms per cubic meter, pg/m^3). The corresponding SCPCS field measurements at San Dimas and Riverside are indicated in brackets. The field data represent only the naphthoquinone collected with PM2.5. The naphthoquinone distribution is quite distinct from that of the parent naphthalene, indicating the important influence of regional dispersion on the concentrations of secondary photochemical products.

Figure 11

Faculty participants in SCPCS research

1. Studies that emphasize investigation of the biological mechanisms of PM effects

Avol, Ed	Exposure assessment, quinone, aldehyde, PAH analysis
Cho, Art	Mechanism, in vitro assays, analytical chemistry
Froines, John	Mechanism
Gong, Henry	Human clinical outcomes, mechanism
Harkema, Jack	Mechanism, in vivo studies
Kleinman, Mike	Mechanism, in vivo studies, in vivo models
Miguel, Toni	Analytical chemistry
Nel, Andre	Mechanism, in vivo, in vitro studies, in vivo models
Paulson, Suzanne	Exposure assessment, peroxides
Phalen, Bob	Dosimetry, mechanism
Sioutas, Costas	Exposure assessment, concentrator development
Stram, Dan	Study design, biostatistics

New Investigators

Fukuto, Jon	In vitro assays, mechanism
Schiestl, Robert	In vitro and in vivo assays, mechanism

2. Source related investigations – Freeway studies/traffic density

Avol, Ed	Exposure assessment
Cho, Art	In vitro assays, analytical chemistry (exposure)
Colome, Steve	Exposure assessment
Froines, John	In vivo assays
Gauderman, Jim	Traffic density/CHS-epidemiology
Hinds, William	Exposure assessment-ultrafines
Kleinman, Mike	In vivo assays, in vivo model development
Lurmann, Fred	Traffic density/CHS-epidemiology
McConnell, Rob	Traffic density/CHS-epidemiology
Miguel, Toni	Analytical chemistry
Nel, Andre	In vitro assays
Nel, Andre	In vivo, in vitro, in vivo model development
Peters, John	Traffic density/CHS-epidemiology
Ritz, Beate	Traffic density/reproductive health-epidemiology
Sioutas, Costas	Exposure assessment, concentrator use
Stram, Dan	Study design, biostatistics

3. Effect of varying spatial and temporal patterns of ambient particles and co-pollutants with particular emphasis on the role of atmospheric chemistry

Colome, Steve	Exposure assessment
Froines, John	In vivo assays
Gong, Henry	Human clinical studies
Harkema, Jack	In vivo assays
Kleinman, Mike	In vivo assays
Lurmann, Fred	Exposure assessment, modeling, CHS
Paulson, Suzanne	Exposure assessment, peroxides
Sioutas, Costas	Exposure assessment, concentrator use
Stram, Dan	Study design, biostatistics
Turco, Rich	Exposure assessment-modeling
Winer, Arthur	Exposure assessment-modeling

4. Data management (overall responsibility)

Colome, Steve
Stram, Dan
Yu, R.C.

Appendix I.

Mechanistic Studies on Airway Inflammation

A major advance since 1997 has been the understanding that airway inflammation contributes to the adverse health effects of PM in the respiratory tract. The elucidation of an inflammatory respiratory effect is compatible with epidemiological data showing a short lag period (1-3 days) between the exposure to elevated PM_{10} and $PM_{2.5}$ levels and onset of morbidity and mortality. While more than one biological mechanism may explain the genesis of airway inflammation, a major finding has been that PM generate reactive oxygen species (ROS), which provide pro-inflammatory stimuli to bronchial epithelial cells and macrophages (Sidebar # 1). These cellular targets respond with cytokine and chemokine production, which enhance the response of the mucosal immune compartment to allergens (Sidebar # 1). PM may therefore act as adjuvants that strengthen the response of the immune system to environmental allergens. The hallmark of allergic inflammation is increased IgE production, eosinophilic bronchial inflammation and airway hyperreactivity. The key role of ROS in the adjuvant effects of PM was indirectly confirmed by the ability of thiol antioxidants to interfere in the allergic inflammatory response to ovalbumin (OVA) in a murine asthma model (Sidebar #1). Human nasal challenge studies confirmed the role of DEP as an adjuvant in already established allergic responses, as well as exposure to neo-allergens. Taken together, these findings may explain the increased number and severity of asthma attacks in an urban setting after a sudden surge in PM levels. The key role of the PM Centers in facilitating this line of investigation has been the promotion of collaboration between scientists with expertise in particle physics (e.g. particle concentrators), animal asthma models, the cellular biology of oxidative stress and inflammation, and inhalational toxicology (Sidebar # 1). Moreover, the collective expertise in the Southern California Particle Center facilitated the use of concentrated air particulates (CAPS) to replace DEP dust for mechanistic in vitro and in vivo studies (Sidebar #1). Animal asthma models are now being used to compare the pro-oxidative and pro-inflammatory effects of CAPS collected on freeways and various source-receptor sites. In addition, human CAPS exposures are now including the role of oxidative stress and airway inflammation, e.g. assays for NO and CO content in the expired air, and measuring cytokines in induced sputum and blood.

Another important development by the EPA Centers is collaboration between organic/analytical chemists, particle engineers and biologists in exploring how chemicals contained in CAPS contribute to ROS generation and inflammation (Sidebars # 1 and 2). An important observation has been that organic components present in the OC fraction generate ROS through their ability to redox cycle in a test tube (Sidebar # 2). These in vitro reactions correlate with the ability of organic PM components to generate oxidative stress in epithelial cells and macrophages. Preliminary evidence indicates that polycyclic aromatic hydrocarbons and their oxidized derivatives (quinones and ketones) play a key role in ROS generation at cellular level. The in vitro toxicity studies predict a hierarchical response, in which the biological effects range from (a) protective (e.g. expression of anti-oxidant enzymes) ? (b) pro-inflammatory (e.g. production of cytokines and chemokines) ? (c) cytotoxic (e.g. cellular apoptosis and necrosis), depending on the level of oxidative stress (Sidebar #1). The ability to relate the inherent redox-cycling and oxidative stress capabilities of a PM sample to specific biological effects allows a more rational interpretation of the in vivo toxicity data generated in community (e.g. Children's Study at

USC), freeway and source/receptor studies. Animal studies are also planned around the principles of the stratified oxidative stress model to ensure that the chosen endpoints are appropriate to the level of exposure and oxidative potential of the particles. Another important application of the stratified response model is the possible identification of susceptible human subjects with weakened oxidative stress defenses. One example is heme oxygenase 1 (HO-1), which is a very sensitive antioxidant defense mechanism that protects cells against redox-cycling DEP chemicals and contributes to CO production during in vivo DEP exposure. The elucidation of susceptible individuals who can be studied with rational endpoints will enhance epidemiological studies and will also help to monitor the impact of regulatory measures on adverse health effects.

Side Bar # 1: Elucidation of Biological Oxidative Stress response pathways through cellular and animal studies

(Collaborators: André Nel, Costas Sioutas, John Froines, Arthur Cho, Robert Devlin)

?? *Target cell types*: Primary bronchial epithelial and epithelial cell lines
Alveolar macrophages and macrophage cell lines

?? *Response origin*: Redox cycling organic chemicals contained in DEP and CAPS ? stratified cellular response (unique profile for epithelial cells/macrophages)

?? *Stratified cellular response characteristics:*

(i) Low stress level ? antioxidant defense pathways
(e.g. heme oxygenase 1, HO-1)

(ii) Intermediate stress level ? pro-inflammatory effects
(e.g. cytokines/chemokines)

(iii) High stress level ? cytotoxic effects
(e.g. apoptosis/necrosis)

?? *Specific intracellular signaling cascades operate at each stress level:*

(i) Low stress level: Activation of anti-oxidant response element in the HO-1 promoter

(ii) Intermediate stress level: Activation of NF-?B and AP-1 response elements in cytokine promoters

(iii) High stress level: Opening of the mitochondrial PT pore and release of apoptogenic proteins

?? *Cellular inflammation* ? adjuvant immune effects ? enhanced IgE production and eosinophilic (allergic) airway inflammation ? reflected by murine asthma models (ovalbumin sensitization) and human nasal challenge studies

?? *Preliminary evidence in animal asthma models for a stratified response* relative to the level of PM exposure

?? *Thiol antioxidants* selectively disrupt oxidative stress and pro-inflammatory effects of DEP in vitro and in vivo

?? Preliminary in vitro evidence that *fine particulates are more potent than coarse particulates* for inducing oxidative stress base on differences in OC and PAH content (higher in fine PM in the summer months)

?? Preliminary evidence has been provided that *PAHs and their oxy-derivatives participate in generating oxidative stress* in epithelial cells and macrophages, e.g. HO-1 expression and cellular toxicity. Chemical fractionation of DEP confirmed that most toxicity reside in the aromatic and polar chemicals.

Sidebar # 2: Chemically-based quantitative toxicity analysis of PM.
(Collaborators: Arthur Cho, John Froines, André Nel, Costas Sioutas, Antonio Miguel)

?? Quantitative analytical procedures for oxidative reactivity have been developed consistent with the hypothesis that organic components contribute to PM toxicity by their ability to generate ROS.

?? Analyses of selected quinones in PM by GC/MS procedures. The results allow comparison of quinone levels across a trajectory of less and more polluted sites in the LA basin.

?? Determination of redox activity in PM by its catalysis of DTT dependent oxygen reduction. This assay provides a measure of the ability of a particular PM sample to generate ROS.

?? Assessment of oxygen dependent and independent toxicity in yeast: establishment of a 50% growth inhibition (IC_{50}) concentration as a quantitative measure of PM toxicity that with the DTT redox assay.